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Encapsulation of curcumin within oil-in-water emulsions prepared by premix
membrane emulsification: Impact of droplet size and carrier oil on the chemical
stability of curcumin

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Abstract

Oil-in-water emulsions containing curcumin with different droplet sizes were produced by premix membrane emulsification with different carrier oils: tributyrin (short chain triglycerides, SCT), medium chain triglycerides (MCT) and corn oil (long chain triglycerides, LCT). The influence of carrier oil type and droplet size on the physical stability, chemical stability of curcumin and lipid oxidation stability of emulsions were investigated. Turbiscan results indicated that the physical stability of emulsions was related to both carrier oils and emulsion droplet sizes. The oil type and droplet size of emulsions stored at 25 °C showed limited effect on the stability of curcumin, but significantly affected the stability of curcumin at 55 °C. Chemical stability of curcumin decreased with the decrease of emulsion droplet sizes. For each droplet size emulsions, the stability of curcumin decreased in the order SCT > MCT > LCT. Moreover, the lipid oxidation in LCT-based emulsions resulted in lower zeta potential of droplets, which was independent of emulsion droplet sizes. The presence of curcumin improved the oxidative stability of emulsions.

Keywords: Curcumin; Emulsions; Droplet size; Carrier oil; Chemical stability; Premix membrane emulsification

Abbreviations: SCT, short chain triglycerides; MCT, medium chain triglycerides; LCT, long chain triglycerides; O/W, oil-in-water; LD, laser diffraction; DLS, dynamic light scattering; A_s , specific surface area; TBARS, thiobarbituric acid-reactive substances; TBA, thiobarbituric acid solution.

1. Introduction

Curcumin is a hydrophobic polyphenol derived from the plant *Curcuma longa* (turmeric). In food, curcumin is widely used as a natural colorant due to its desirable yellow-orange color. Moreover, curcumin is a food additive (E100) approved by the European Union and is well known for its wide range of beneficial activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, antiviral and wound healing (Maheshwari et al., 2006; Zorofchian Moghadamtousi et al., 2014; Akbik et al., 2014; Jiang et al., 2020). In recent years, there has been a strong interest in the food industries into the potential utilization of curcumin as a nutraceutical in functional foods and supplements products because of its potential health benefits. However, curcumin is insoluble in water and highly sensitive to environmental stresses during food processing and storage, such as heat, light, and oxygen, which limited its application as a nutraceutical. To overcome these limitations, many colloidal delivery systems has been explored for improving the water solubility, stability and bioavailability of curcumin, including emulsions (Kharat et al., 2018; Jiang & Charcosset, 2022b), liposomes (Peng et al., 2017; Wu et al., 2020) and nanoparticles (Zhang et al., 2021; Chen et al., 2022).

Oil-in-water (O/W) emulsions consisting of an aqueous phase, surfactants, and carrier oil have been widely used to encapsulate and protect curcumin (Kharat et al., 2018, 2020; Jiang & Charcosset, 2022b). In these systems, curcumin is solubilized inside the oil droplets and/or the surface layer of the oil droplets (Cheng et al., 2020), which protects curcumin from chemical degradation by avoiding the reaction with the active substances within the aqueous phase. Nevertheless, the composition and structure of emulsions, such as surfactant type, carrier oil type, droplet size and pH, significantly influence the chemical stability of encapsulated curcumin. Kharat et al. (2018) investigated the impact of surfactant type (sodium caseinate, Tween 80, quillaja saponin, gum arabic) and concentration (critical and excess) on the chemical stability of curcumin in emulsions. They observed that emulsions prepared using saponin had

relatively poor stability to curcumin degradation due to the fact that saponins containing conjugated double bonds generate free radicals that promote peroxidation reactions, while the other three emulsifiers behave fairly similarly. Moreover, they found that the stability of curcumin in emulsions was not significantly influenced by the concentration of surfactant. Recently, Kharat et al. (2020) fabricated curcumin-loaded emulsions with different droplet sizes and found that the stability increased with increasing emulsion droplet sizes because large droplets have smaller surface area that led to a slower exchange of curcumin molecules between the oil and water phase. Carrier oil type may play an important role in determining chemical stability of curcumin. However, the effect of carrier oil type and the underlying mechanisms are not well studied. Normally, curcumin is primarily located within the oil phase of emulsions. Nevertheless, some curcumin molecules are also present in the surface layer of oil droplets and/or in the aqueous phase depending on the solubility of curcumin in oils, which may have an influence on the chemical stability of curcumin. Moreover, unsaturated oils are more susceptible to oxidation, which may affect the stability of curcumin.

The generation of emulsions can be achieved through different processes such as phase inversion temperature, phase inversion composition, ultrasonication, high pressure homogenization and microfluidization (Jiang et al., 2020). Premix membrane emulsification is a more recent process to produce emulsions using mild mechanical forces but with lower energy input compared to other emulsification methods (Vladislavljevic et al., 2004; Nazir et al., 2010). In premix membrane emulsification, large droplets of coarse premix are disrupted into fine droplets by internal shear in the pores caused by flow through a microporous membrane as a special kind of low-pressure homogenizing valve (Vladislavljevic et al., 2004). Mono-dispersed O/W emulsions with controlled droplet sizes and narrow droplet size distributions can be prepared by premix membrane emulsification with hydrophilic Shirasu Porous Glass (SPG) membranes with different pore size (Alliod et al., 2018; Jiang & Charcosset, 2022a).

In the present work, three carrier oils were selected that have different molecular characteristics and curcumin solubility: tributyrin, medium chain triglycerides (MCT) and corn oil. Tributyrin was used as an example of short chain triglycerides (SCT), and corn oil is an example of unsaturated long chain triglycerides (LCT). We aim to obtain O/W emulsions with different droplet sizes for SCT, MCT and LCT, respectively, by premix membrane emulsification, and to study the influence of emulsion droplet size and carrier oil on the physical stability, chemical stability of curcumin and lipid oxidation stability of emulsions.

2. Materials and methods

2.1. Materials

Tween 80, Span 80, curcumin ($\geq 99.5\%$), curcumin analytical standard ($\geq 98.0\%$), acetic acid of HPLC grade, tributyrin (SCT oil), corn oil (LCT oil), 2-propanol, ammonium thiocyanate, iron (II) chloride, iron (III) chloride, 2-thiobarbituric acid, trichloroacetic acid, hydrochloric acid, chloroform, and 1,1,3,3-tetramethoxypropane were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol of HPLC grade were purchased from Carlo Erba. LabrafacTM lipophilic WL 1349 (MCT oil) was obtained by an esterification reaction between glycerol and caprylic/capric acids (C8-C10), and kindly provided by Gattefossé (Saint-Priest, France). 1-butanol was purchased from Fisher Chemical. Isooctane was obtained from Alfa Aesar.

2.2. Preparation of curcumin emulsions

O/W emulsions with different carrier oils and droplet sizes containing curcumin were obtained by premix membrane emulsification as described in our previous article (Jiang & Charcosset, 2022a). The preparation process to obtain emulsions was exactly the same with our other previous article (Jiang & Charcosset, 2022b). Emulsions with different droplet sizes were produced using membranes with different pore sizes: 0.4 μm (small emulsions), 0.8 μm (medium emulsions) and 3.1 μm (large emulsions). All experiments were carried out with 100

mL premix. After preparation, all emulsions were stored at 4 °C.

2.3. Characterization of curcumin-loaded emulsions

2.3.1. Droplet size, zeta potential and specific surface area

Droplet size of emulsions were determined by laser diffraction (LD) particle size analysis with a Mastersizer 3000 (Malvern Instruments, UK). D[4,3] and D[3,2] values representing volume mean diameters and surface area mean diameters, respectively, were obtained. In addition, droplet size of small and medium emulsions and the electrical characteristics of the oil droplets (zeta potential) were measured using a Zetasizer Nano ZS (Malvern Instruments, UK). The droplet size was determined using dynamic light scattering (DLS), at a wavelength of 633 nm and scattering angle of 173° at 25°C. Zeta potential was measured by the principle of electrophoresis using Doppler Effect and calculated based on Smoluchowski model (Sze et al., 2003). All samples were diluted with 5 mM phosphate buffer (pH 7.0) (the dilution factor was adjusted to obtain an attenuation factor between 5 and 7) before analysis to avoid multiple scattering effects. All droplet size measurements were performed after emulsions were redispersed using a magnetic stirrer at 300 rpm for 1 min at room temperature. The specific surface area (A_S) of the oil droplets in the emulsions, which is the oil-water interfacial area per unit mass of oil ($\text{m}^2 \text{kg}^{-1}$), was calculated using the following expression (Kharat et al., 2020) (1):

$$A_S = 6 / (\rho_0 D[3,2]) \quad (1)$$

where ρ_0 is the density of the oil phase (1032 kg m^{-3} , 940 kg m^{-3} and 920 kg m^{-3} for SCT, MCT and LCT, respectively) and $D[3,2]$ is the surface-weighted mean droplet diameter (m).

2.3.2. Viscosity

Shear viscosity measurements were performed using a controlled stress rheometer (MCR 302 Rheometer, Anton Paar Gmbh, Graz, Austria), equipped with a cone-and-plate geometry (12.490 mm cone diameter, 0.978 cone angle, 49 μm gap). All measurements were carried out

at shear rate ranging from 0.01 to 100 s⁻¹, with temperature controlled at 25.0 ± 0.1 °C.

2.4. Turbiscan analysis

The creaming and sedimentation stability of emulsions was monitored by a TurbiscanTM Classic MA 2000 (Formulaction Inc., Toulouse, France). Undiluted emulsions (approximately 6 mL) were placed in a Turbiscan special cylindrical glass tube at 25 °C. Small emulsions were scanned every 6 h for 72 h, medium emulsions every 4 h for 48 h, large emulsions every 1 h for 12 h, and premix emulsions every 2 min for 24 min. The data of back scattering (%) from emulsions as a function of their height over the measurement time were acquired.

2.5. Stability study

To evaluate the impact of droplet size and carrier oil type on emulsions storage stability, samples were stored in glass bottles at 25°C for 60 days and 55 °C for 12 days in the dark. At selected times, samples were taken to measure the chemical stability of curcumin. The appearance of emulsions was also visually observed.

2.5.1. Chemical stability of curcumin

Curcumin-loaded emulsions were subjected to different heat and storage conditions, which resulted in mass loss of curcumin. Chemical stability of curcumin was assessed by measuring the change of curcumin retention rate. At scheduled time, 0.5 mL of sample was collected, diluted with ethanol (1:5 (v%)) and centrifuged at 5000 g for 15 min at 25°C. The supernatant was then analyzed for curcumin concentration using an Agilent 1200 HPLC system (Agilent Technologies Inc., USA) with UV detector (UV lamp and vis lamp), a Phenomenex C18 column (100 mm×4.6 mm, 2.6 μm) and Agilent ChemStation software. The mobile phase was composed of acetonitrile and 0.6% (v%) aqueous acetic acid at ratio of 40/60% (v%), the flow rate was 1 mL/min with a column temperature of 35°C, and an injection volume of 10 μL. Curcumin was detected at a wavelength of 425 nm. The calibration curve was established based on the relationship between the HPLC peak area (y) and curcumin concentration (μg/mL) (x),

which is $y = 69.443x - 59.782$ ($R^2 = 0.9998$). Quantification of curcumin was performed using the calibration curve. Curcumin chemical stability (retention rate) was calculated by the following equation (2):

$$\text{Retention rate}(\%) = \frac{\text{Content of curcumin (t)}}{\text{Total amount of curcumin (t=0)}} \times 100 \quad (2)$$

where the total amount of curcumin is the initial content of curcumin, and the content of curcumin is the curcumin amount that was measured at different sampling time.

2.5.2. Lipid oxidation

2.5.2.1. Primary oxidation compounds

Lipid hydroperoxides were determined by the international dairy federation (IDF) method as described by Shanta and Decker (1994), with some modification. Briefly, lipid extraction was performed by adding 0.3 mL of emulsions to a mixture of 1.5 mL isooctane/2-propanol (3:2 v/v) and then vortexing the sample for 30 s, followed by centrifugation at 1000 g for 2 min. Next, 0.1 mL of the upper organic phase (0.9 mL total volume) was diluted 10 times and then 0.1 mL of diluted solution mixed with 1.4 mL of pre-mixed methanol/1-butanol (2:1 v/v), followed by the addition of 50 μL of 3.94 M ammonium thiocyanate and 50 μL of 0.132 M ferrous iron solution. The absorbance was measured at 500 nm after 20 min using an UV5 visible spectrophotometer (Mettler Toledo, Columbus Ohio, USA). Lipid hydroperoxides concentrations were determined using a Fe^{3+} standard curve (0-100 $\mu\text{g/mL}$). The peroxide value expressed as millequivalents (meq) of peroxide per kilogram of sample using the following equation (3):

$$\text{peroxide value} = (A_S - A_B) / (m \times 55.84 \times m_0 \times 2) \times 90 \quad (3)$$

where A_S is the absorbance of the sample, A_B is the absorbance of the blank, m is the slope obtained from the calibration curve, m_0 is the mass of the sample (g), 55.84 is the atomic weight of iron (g/mol), 2 is a division factor necessary to express the peroxide value as milliequivalents

of peroxide instead of milliequivalents of oxygen, 90 corresponds to the inverse of the dilution factor related to the isooctane organic phase (0.9/0.1*10).

2.5.2.2. Secondary oxidation compounds

Thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, were measured according the method reported by McDonald and Hultin (1987). Firstly, thiobarbituric acid solution (TBA) was prepared by dissolving 0.09375 g of thiobarbituric acid, 3.75 g of trichloroacetic acid in 25 ml of 0.25 M HCl. Then, 0.5 mL of emulsions was mixed with 1 mL of TBA reagent in test tubes and placed in a boiling water bath for 15 min. After that, the tubes were cooled to room temperature for 10 min and then treated with 0.3 mL chloroform and centrifuged at 3000 g for 15 min. The absorbance of the supernatant was measured at 532 nm (Mettler Toledo, Columbus Ohio, USA). TBARS concentrations were calculated using a standard curve prepared using 1,1,3,3-tetramethoxypropane (0-30 μ M).

2.6. Statistical analysis

The stability experiments were performed in duplicate, and all measurements were performed in duplicate. The results were expressed as the means \pm standard deviation (SD), and statistical analyses were performed with SPSS17.0. The statistical comparisons were conducted using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) and Duncan test. In all cases, $p < 0.05$ was regarded statistically significant.

3. Results and discussion

3.1. Characterization of curcumin-loaded emulsions

For each carrier oil, curcumin-loaded emulsions with three different droplet sizes were produced by premix membrane emulsification using 0.4 μ m, 0.8 μ m and 3.1 μ m pore size membranes. The coarse emulsions were prepared by magnetic stirrer and were not further processed by premix membrane emulsification. They were used as premix. The resulting pressure in the preparation process, emulsions droplet sizes, specific surface area and emulsion

viscosity of four different droplet size emulsions for each oil are summarized in **Table 1**. The resulting pressure is an important factor in premix membrane emulsification that has to be lower than 60-80 bar to avoid membrane rupture. As explained in our previous article (Jiang & Charcosset, 2022a), the resulting pressure in the preparation process is equal to the sum of the pressure needed for droplet disruption (disruption pressure), pressure due to flow of the premix through the membrane pores (flow pressure) and pressure in the pipes from the pump to the membrane module (pipe pressure). In our experiments, the flow rate was set at 200 mL/min to prepare emulsions when using the 0.8 μm and 3.1 μm pore size membranes. The flow rate had to be reduced to 50 mL/min for the 0.4 μm pore size membrane as a lower flow rate resulted in a lower pressure protecting membrane from rupture. We found that the resulting pressure with the 0.8 μm pore size membrane was higher than that with the 3.1 μm pore size membrane when the flow rate was set at 200 mL/min for three different carrier oil-based emulsions. Moreover, the resulting pressure observed with the 0.4 μm pore size membrane using a flow rate of 50 mL/min was higher than that with the 0.8 μm and 3.1 μm pore size membranes at a flow rate of 200 mL/min. This can be explained by the fact that small pore size membranes require higher disruption pressure for extrusion to produce smaller droplets (Jiang & Charcosset, 2022a). In addition, it was found that the carrier oils had a great impact on the resulting pressure. The resulting pressure increased in the order of SCT < MCT < LCT when the same pore size membrane was applied to prepare emulsions. This may be caused by the different viscosity of carrier oils resulting in different viscosity of the premix (3.25 ± 0.30 , 4.27 ± 0.55 and 6.12 ± 0.26 mPa·s for SCT, MCT and LCT premix, respectively), which affects the flow pressure then the resulting pressure.

For all three carriers oil-based emulsions, the viscosity of premix was significantly ($p < 0.05$) higher than that for small, medium and large emulsions, while there was no significant ($p > 0.05$) difference among the viscosity of small, medium and large emulsions. The surface area

decreased with the increase of emulsion droplet sizes, which means that premix emulsions needed less surfactants molecules compare to small, medium and large emulsions while all emulsions had the same concentration of surfactant. This made more surfactants molecules dissolved in aqueous phase leading to a higher viscosity.

The droplet sizes of small and medium emulsions obtained from DLS were close to the membrane pore size, which is in agreement with our previous studies (Alliod et al., 2018; Jiang & Charcosset, 2022a). In LD particle size analysis, obtained $D[4,3]$ and $D[3,2]$ values for small, medium and large MCT and LCT emulsions were slightly higher than the pore size of membrane (**Table 1**), which can be explained by the fact that volume distribution used in LD analysis leads to higher droplet sizes than in DLS analysis. However, for small, medium and large SCT emulsions, both $D[4,3]$ and $D[3,2]$ values were lower than the membrane pore size (**Table 1**). For SCT emulsions, the difference in droplet size values in DLS and LD analysis were rather large. This may be attributed to the fact that intensity distribution and volume distribution were used in DLS and LD analysis, respectively, which may have an impact on the droplet size results. Droplet size distribution of emulsion with different droplet sizes and different carrier oils by intensity and volume were shown in **Fig. S1**. The size distribution by intensity for three carrier oils emulsions were similar in small and medium emulsions, while size distribution by volume for SCT emulsions moved to left compared to MCT and LCT emulsions for all small, medium and large emulsions. The differences in size distribution and droplet size values in LD analysis is probably due to the fact that SCT has a relatively higher water solubility compared to MCT and LCT, which may influence the dissolution of SCT droplets leading to smaller droplet size. $D[4,3]$ values of droplets in SCT, MCT and LCT premixes were $59.7 \pm 1.1 \mu\text{m}$, $56.4 \pm 0.60 \mu\text{m}$ and $64.6 \pm 0.94 \mu\text{m}$ (**Table 1**), respectively. $D[3,2]$ values were $42.9 \pm 1.06 \mu\text{m}$, $26.85 \pm 0.90 \mu\text{m}$ and $36.5 \pm 0.64 \mu\text{m}$, respectively. In order to calculate the As of the oil droplets in the emulsions, $D[3,2]$ values of droplets in SCT, MCT

and LCT premix were taken even if $D[3,2]$ values did not show the greatest sensitivity in the large particle fraction. For MCT and LCT emulsions, the calculated A_s values were approximately 14 000, 7 000, 2 000, and 200 $\text{m}^2 \text{kg}^{-1}$ for the small, medium, large, and premix emulsions, respectively (**Table 1**). The calculated A_s values for small, medium and large SCT emulsions were about 31 000, 9 000 and 3 000 $\text{m}^2 \text{kg}^{-1}$, respectively, which were higher than that of MCT and LCT emulsions prepared with the same membrane pore size due to the smaller droplet size values of SCT emulsions obtained from LD analysis.

3.2. Physical stability analysis

The Turbiscan technology, which is commonly used to evaluate the stability of colloidal dispersions, was used in the present study to investigate the physical stability of different droplet sizes (small, medium, large, and premix) SCT, MCT and LCT emulsions. The processes of destabilizing emulsions can be detected by a non-homogeneous variation of back scattering. The back scattering spectra of emulsions with different droplet sizes for each oil at room temperature are shown in **Fig. 1**. For SCT-based emulsions, the back scattering values of emulsions with small and medium droplet sizes were almost constant within 72 h and 48 h respectively (**Fig. 1A**), which means that samples were homogeneous with good physical stability. The back scattering values of emulsions with large droplets showed little changes in the middle of the sample, while the values increased with time at the bottom and decreased with time at the top of the sample (**Fig. 1A**). The increase of back scattering intensity at the bottom of the samples and the reduction of back scattering intensity at the top indicated droplets sedimentation. However, the back scattering values of premix SCT emulsions were sharply increased at the bottom and decreased at the top of the sample (**Fig. 1A**), reflecting the increased turbidity at the bottom, that is to say, sedimentation. The occurrence of sedimentation is due to the fact that density of oil phase (1.032 g/m^3) is higher than that of water phase (0.997 g/m^3). Therefore, we can conclude that the physical stability of emulsions decreased with the increase

of emulsions droplet sizes. On the other hand, it was observed that the back scattering values of different size emulsions obtained in the beginning (at time zero) decreased with the increase of droplet sizes. This is due to the fact that droplet population density decreased with the increase in emulsion droplet sizes resulting in lower back scattering of light (Shirasangi et al., 2020).

For MCT-based emulsions, the back scattering values of emulsions with small droplet size were almost constant within observation time, while varying degrees of changes of the back scattering values in medium, large and premix emulsions were observed (**Fig. 1B**). In medium, large and premix emulsions, the back scattering values decreased at the bottom of the samples and increased at the top, which suggests destabilization by cream formation. The occurrence of creaming was caused by movement of droplets in emulsions because of the density difference between oil phase (0.93-0.96 g/m³) and water phase (0.997 g/m³).

For LCT-based emulsions, the back scattering values changes in the four different droplet sizes emulsions had similar trend with that in different size MCT-based emulsions (**Fig. 1C**), which suggested that creaming occurred in LCT-based emulsions. However, the change of back scattering values in 72 h in small droplet size LCT-based emulsions was bigger than that in small droplet size MCT-based emulsions, and larger changes were observed as well in medium and large droplet size LCT-based emulsions compared to that in medium and large droplet size MCT-based emulsions, respectively. This can be explained by the density difference between MCT (0.93-0.96 g/m³) and LCT (0.91-0.93 g/m³). The density difference between LCT and water is higher than that between SCT and water, which led to a lower physical stability in LCT-based emulsions at the same conditions. Coalescence and Ostwald ripening are characterized by a decrease in the back scattering values over the entire length of the sample (Zheng et al., 2018), which did not occur for all the emulsions studied, indicating that all droplet sizes emulsions were stable during storage.

The above results showed that the physical stability of emulsions was affected by the droplet size and oil type. The destabilization rate was slower in the emulsions containing small droplets, which is caused by droplet interaction and crowding effect (Chanamai & McClements, 2000). Normally, the number density of droplets increased with a decrease in droplet size of emulsion. The density difference between dispersed and continuous phases has a great impact on emulsions physical stability because of gravity effect. A relatively small density difference between oil and water can help to form emulsions with a better physical stability.

3.3. Influence of oil type and emulsions droplet size on emulsions appearance

At the beginning of storage, all curcumin-loaded emulsions had a milky yellow appearance, and the three premix emulsions showed deeper yellow color because of their lower encapsulation efficiency as explained in our previous article (Jiang & Charcosset, 2022b) (**Fig. 2**). Moreover, the premix emulsions were less turbid than others, corresponding to the results of Turbiscan analysis, which is attributed to the reduced light scattering associated with larger droplets.

After 8 weeks of storage at room temperature, the oil droplets in medium, large and premix SCT-based emulsions had moved to the bottom of the tube, forming a yellow sediment layer under a transparent layer (**Fig. 2A**). On the contrary, for MCT and LCT emulsions, medium, large and premix emulsions separated into a yellow creamy layer on the top of a clear layer (**Fig. 2A**). Sedimentation and creaming occurred because of the density difference between the dispersed and continuous phases and the gravitational separation. In order to observe the color change of emulsions, photographs of redispersed emulsions are also shown. As we can see from **Fig. 2A**, the change in color for all emulsions compared to the emulsions at the beginning of storage was rather low.

After 12 days of storage at 55°C, creaming occurred in MCT and LCT emulsions, similarly to the emulsions stored at room temperature for 8 weeks, while sedimentation phenomenon had

not occurred in small, medium and large SCT emulsions probably due to the fact that the rate of Brownian motion increased at 55°C led to a better physical stability (**Fig. 2B**). However, we observed that the intensity of the yellow color decreased between the initial emulsions before storage and redispersed emulsions after storage for all emulsions, which indicates chemical degradation of curcumin. Moreover, we found that oil type had an impact on the intensity of the yellow color of emulsions after 12 days storage at 55°C. As seen in **Fig. 2B**, the intensity of the yellow color of emulsions decreased in the order of SCT > MCT > LCT. However, little changes in color intensity were observed for the different size emulsions. Therefore, curcumin retention rates were calculated in next section to study the influence of emulsions droplet size on curcumin chemical stability.

3.4. Influence of oil type and emulsions droplet size on the chemical stability of curcumin

The influence of the oil type and emulsions droplet size on the chemical stability of curcumin was investigated at 25°C and 55°C. The change of curcumin retention rate over time during storage was used to assess curcumin chemical stability.

At 25°C, curcumin retention rate for all emulsions decreased slowly during storage periods (**Fig. 3A, 3B and 3C**). Curcumin retention rates changed very slowly due to the slow oxidation and hydrolysis reaction at room temperature. After 8 weeks, curcumin retention rates were in the range of 83% to 95% (**Table 1**), corresponding to the high intensity of yellow color at the end of storage which was observed in **Fig. 2A**. Except curcumin retention rates in small MCT-based emulsions significant lower ($p < 0.05$) than that in other size emulsions, there was no significant difference ($p > 0.05$) in curcumin retention rates for all droplet size emulsions for the same carrier oil (**Table 1**). It seems that all emulsions showed similar curcumin stability at room temperature.

There was a relatively rapid decrease in curcumin retention rates during storage at 55°C for all emulsions (**Fig. 3D, 3E and 3F**). For SCT and MCT-based emulsions, curcumin retention rates

increased as the droplet size increased at all measurement times (**Fig. 3D and 3E**). At the end of storage, there was a significant difference ($p < 0.05$) in curcumin retention rates among small, medium and premix emulsions (or small, large and premix emulsions), while no significant difference ($p > 0.05$) was observed for medium and large emulsions (**Table 1**). Overall, curcumin stability in SCT and MCT-based emulsions decreased with the decreased emulsion droplet sizes because of the increased surface area, which is consistent with an earlier study (Kharat et al., 2020). For LCT-based emulsions, curcumin retention rates in the premix emulsion decreased gradually during storage, while that in small, medium and large emulsions decreased gradually during 9 days but then kept almost constant (**Fig. 3F**). This relatively constant value can be explained by the fact that the HPLC peak areas were about 0 at 9 days which means that almost all curcumin was degraded, and then the calculated retention rate at 12 days was almost the same with that at 9 days. At the end of storage, curcumin retention rate in the premix emulsions were significantly ($p < 0.05$) higher than that in the small, medium and large emulsions. On the other hand, it is important to note that the creaming and sedimentation phenomenon in medium, large and premix emulsions may have reduced the exposure of the oil phase to the surrounding water phase, thereby reducing the exchange rate of curcumin molecules between the oil and the aqueous phases, which may have an impact on the curcumin stability. In contrast, small emulsions were relatively more stable, which means that oil droplets remained dispersed in the aqueous phase during storage.

Moreover, it was found that the carrier oil type had a great impact on curcumin stability. For each droplet size emulsions, the curcumin retention rate decreased in the order of SCT > MCT > LCT (**Table 1**), corresponding to the observation in **Fig. 2B**. The lowest curcumin retention rate in LCT-based emulsions is due to the fact that LCT is an unsaturated oil (Zhou et al., 2018), which is particularly susceptible to lipid oxidation resulting in the fastest degradation of curcumin. Both SCT and MCT are saturated oils, but curcumin retention rate in SCT was higher

than that in MCT. This may suggest that curcumin stability is related to the partition of curcumin in the inner oil phase and the surface layer. Cheng et al. (2020) reported that the partition of bioactive compounds in the whole emulsion was dependent on their solubility in the oil phase and surfactant layer. In our previous study (Jiang & Charcosset, 2022b), the solubility of curcumin in three different oils and two surfactants were investigated. It was found that solubility in SCT was nearly three times as much as that in MCT and five times as much as in LCT. The highest solubility in SCT suggested highest percentage of curcumin in the inner oil phase and lowest percentage of curcumin in the surface layer of oil droplets. Therefore, the higher retention rate of curcumin in SCT-based emulsions can probably be explained by the fact that a higher percentage of curcumin was encapsulated in the inner oil phase, which protected curcumin from sensitive environment as explained by Cheng et al. (2020), who studied the stability of resveratrol in different oil emulsions.

3.5. Influence of emulsions droplet size and curcumin on zeta potential

Both the emulsions droplet sizes and zeta potential were measured before and after storage at 25°C and 55°C. It was found that there was no significant change in the droplet sizes of all emulsions during storage at 25°C and 55°C (data not shown), indicating that all emulsions were resistant to these two temperatures. Initially, the zeta potential for all emulsions was negatively charged (-18 to -21 mV) (**Fig. 4**), and did not change significantly ($p > 0.05$) regardless of the droplet size and oil type. Even though it was not expected to have any charge in emulsions because Tween 80 and Span 80 are nonionic surfactants, it is possible to have negative charges due to the presence of ionizable surface active impurities and phosphate buffer (McClements, 2004). No significant ($p > 0.05$) decrease in zeta potential was observed for emulsions with decreasing droplet sizes, which was in disagreement with the results of Kharat et al. (2020), who found that the magnitude of the zeta potential decreased with decreasing droplet sizes in saponins-stabilized emulsions. This effect was attributed to the different surfactants. Saponins

have a strong negative charge at pH 7.0 (the studied pH), which changed droplet surface chemistry, while Tween 80 and Span 80 do not have any charges. After storage at 25°C for 8 weeks, no significant change ($p > 0.05$) was observed in the zeta potential of all emulsions (data not shown). At 55°C, the zeta potential did not significant change ($p > 0.05$) for SCT and MCT emulsions after 12 days (**Fig. 4A** and **4B**). However, the zeta potential of all LCT emulsions at 12 days slightly decreased compared to the values obtained at the beginning of storage (**Fig. 4C**). The decrease of zeta potential could be due to changes in the surface chemistry of the lipid droplets, that may result from the generation of charged reaction species caused by curcumin chemical degradation or lipid oxidation. In order to identify the physicochemical origin of the interfacial changes, LCT-based emulsions in the absence of curcumin (blank emulsions) were prepared, and their zeta potential were studied before and after storage. It has been found that the absence of curcumin led to a lower zeta potential compared to the zeta potential of curcumin-loaded emulsions at 12 days (**Fig. 4C** and **4D**), indicating that the products of lipid oxidation changed the surface chemistry of the lipid droplets. Therefore, the presence of curcumin had a great impact on lipid oxidation, which is discussed in next section.

3.6. Influence of emulsion droplet size and curcumin on LCT oxidation

Lipid oxidation occurs during storage, and the formation of oxidation products affects product quality as it leads to changes in flavor and nutrient value (Ahmed et al., 2016). To evaluate the effect of emulsions droplet size and curcumin on emulsions oxidative stability, both primary and secondary oxidation products were measured periodically when emulsions were stored at 55°C for 12 days. The oxidation reactions first lead to the formation of hydroperoxide, which further forms secondary oxidation products, such as ketones and aldehydes. The peroxide value determines the amount of hydroperoxides, and TBARS detects the level of malondialdehyde, the major lipid oxidation product.

For SCT and MCT emulsions, there were no changes in the peroxide values and TBARS during

storage at 55°C for all droplet size emulsions (peroxide and TBARS values were about 10 meq/kg sample and 1µM, respectively). This can be explained by the fact that both SCT and MCT are saturated oils, which are very stable and not easily oxidized. However, LCT contains 59% polyunsaturated fatty acid and 24% monounsaturated fatty acid (Dupont et al., 1990) that are much readily oxidized. Oxidation stability of LCT emulsions with and without curcumin during storage at 55°C are illustrated in **Fig. 5**. The initial peroxide value for all droplet size emulsions with or without curcumin were at a relatively low level (**Fig. 5A** and **5B**). The peroxide value of all droplet size emulsions increased gradually, except for large and premix emulsions without curcumin stored at 55°C for 12 days, indicating that lipid oxidation occurred during storage. Interestingly, the peroxide value of the large and premix emulsions without curcumin increased until 6 days and then decreased slightly. A similar phenomenon was reported by Liu et al. (2019), who observed the decline of peroxide value of peanuts when stored at 35°C for 320 days. This phenomenon may be attributed to the fact that the initial steps of lipid oxidation involve chain reactions that form unstable hydroperoxides, which decompose into secondary oxidation products (Andersen & Skibsted, 2002; Liu et al., 2019). Additionally, it has been found that in 12 days of storage at 55°C, the peroxide value had an irregular behavior with the change of emulsion droplet size, indicating that this parameter was independent of emulsion droplet size (**Fig. 5A** and **5B**). This can be explained by the limited oxygen present in the sample, the amounts of primary oxidation products were similar whatever the droplet sizes of emulsions (Lethuaut et al., 2002). This result is in agreement with previous studies that investigated the effect of emulsion droplet size on lipid oxidation (Osborn & Akoh, 2004; Walker et al., 2015; Roppongi et al., 2021). However, some studies reported that increasing the emulsion droplet sizes led to a better oxidative stability of emulsions due to the decrease in surface area of droplets, which is exposed to the aqueous phase (Azuma et al., 2009; Yalçınöz & Erçelebi, 2020). Moreover, some papers report a better oxidative stability of O/W emulsions

with smaller droplets than with larger ones (Nakaya et al., 2005; Kikuchi et al., 2014). These studies are not consistent with our results. Further investigations are needed to better understand the effect of emulsion droplet size on lipid oxidation. Moreover, it has been found that curcumin in emulsions has an effect on the peroxide value of emulsions (0.1% w/w curcumin in LCT) (**Fig. 5A** and **5B**). Although the general trends were fairly similar, the peroxide value in the emulsions without curcumin were appreciably higher than those in the emulsions with curcumin at the same storage time. For example, by the end of storage, the peroxide values were lower than 250 meq/kg sample in all curcumin-loaded emulsions, while the peroxide values almost reached 400 meq/kg sample in small, medium and large emulsions in absence of curcumin. Therefore, the emulsions without curcumin were less oxidatively stable than curcumin-loaded emulsions, which suggests that curcumin has antioxidant activity protecting the oil from oxidation.

Fig. 5C and **5D** show TBARS of emulsions in presence or in absence of curcumin, respectively, during storage at 55°C. Similarly to the hydroperoxide results, the level of TBARS formed in all emulsions increased gradually, except for premix emulsions without curcumin stored at 55°C for 12 days. The decrease in TBARS values in premix emulsions without curcumin after 9 days of storage may be due to the formation of other secondary products of lipid oxidation which do not react with the TBA reagent (Ulbin-Figlewicz & Jarmoluk, 2016). Emulsion droplet size did not affect the TBARS levels, which is in agreement with the hydroperoxide results discussed previously. Moreover, we found that the presence of curcumin inhibited the formation of TBARS, which is probably due to the antioxidant activity of curcumin.

Overall, our study indicates that lipid oxidation was independent of initial emulsion droplet sizes. However, there is still some controversy regarding the effect of emulsion droplet size on lipid oxidation. These conflicting results obtained with different dispersed systems, and various interpretations by authors, underline the fact that droplet size and interfacial area probably affect

lipid oxidation in emulsions through several mechanisms depending on various structural and compositional parameters of these colloidal systems. The underlying mechanisms are not well understood. In addition, the results suggest that the presence of curcumin improved the oxidative stability of all emulsions, as demonstrated by its ability to inhibit the formation of hydroperoxides and TBARS. The inhibitory effect of curcumin on lipid oxidation can be attributed to its chemical reactivity. The phenolic hydroxyl groups or the methylene group of the β -diketone moiety in curcumin can undergo a redox transition, involving a transfer of one electron or hydrogen atom to a free radical, to scavenge radicals and consequently produce a phenoxyl radicals or the carbon-centered radical (**Fig. S2A**). Furthermore, the phenolic hydroxyl groups in curcumin are first converted to a phenolic radical, which then migrates to the conjugated heptadienedione chain and initiates a chain reaction of curcumin degradation to generate various autoxidation compounds (**Fig. S2B**).

4. Conclusions

This study has extensively investigated the impact of carrier oil type and initial emulsions droplet size on the colloidal physical stability, curcumin chemical stability and lipid oxidation stability in O/W emulsions. Turbiscan results showed that carrier oil type and initial emulsions droplet size affected the physical stability of emulsions. The stability of emulsions increased with the decrease of emulsions droplet sizes for the three carrier oils, since the droplet interaction and crowding effect increase with the number density of droplets. In addition, this study has shown that the chemical stability of curcumin was independent of emulsion droplets size and carrier oil during storage at room temperature, while emulsion droplets size and carrier oil played a critical role in determining the chemical stability of curcumin during storage at 55°C. At 55°C, the chemical stability of curcumin decreased with the decrease of emulsion droplet sizes, which is attributed to the increase in surface area of oil exposed to the surrounding aqueous phase. For each droplet size emulsions, curcumin stability decreased in the order of

SCT > MCT > LCT. The highest stability of curcumin in SCT-based emulsions is due to the fact that SCT has the highest curcumin solubility, so curcumin was encapsulated in the inner oil phase, protecting curcumin from sensitive environment. The lowest curcumin stability in LCT-based emulsions is due to the fact that LCT is an unsaturated oil, which is particularly sensitive to lipid oxidation resulting in the fastest degradation of curcumin. Moreover, it was found that lipid oxidation in LCT-based emulsions was independent of initial emulsion droplet sizes, which can be explained by the limited oxygen present in the samples during storage. The presence of curcumin improved the oxidative stability of all droplet size emulsions, which confirmed the antioxidant activity of curcumin. These results have important implications for the food and pharmaceutical industries to design and fabrication of emulsions-based delivery systems for curcumin and other lipophilic functional ingredients, to choose better processing and storage conditions.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Table 1

The resulting pressure in premix membrane emulsification, droplet size, viscosity of different droplet size curcumin-loaded emulsions containing different oil phase, the retention rate of curcumin after 12 days at 55°C and 8 weeks at 25°C.

Carrier oil	Membrane pore size (μm)	Pressure (bar)	Z-Average (nm)	D[4,3] (μm)	D[3,2] (μm)	Surface area (m ² kg ⁻¹)	Viscosity (mPa·s)
SCT	0.4 (Small)	33.8 ± 2.5	463 ± 12	0.29 ± 0.02	0.19 ± 0.02	31234 ± 3237	1.58 ± 0.03 ^b
	0.8 (Medium)	12.8 ± 0.5	857 ± 28	0.66 ± 0.04	0.63 ± 0.02	9224 ± 10	1.60 ± 0.13 ^b
	3.1 (Large)	2.8 ± 0.1	-	2.30 ± 0.09	1.77 ± 0.07	3283 ± 127	1.83 ± 0.23 ^b
	Premix	-	-	59.7 ± 1.1	42.9 ± 1.06	136 ± 4	3.25 ± 0.30 ^a
MCT	0.4 (Small)	39.8 ± 1.7	494 ± 5	0.49 ± 0.01	0.46 ± 0.01	13985 ± 421	1.65 ± 0.12 ^b
	0.8 (Medium)	16.7 ± 1.4*	823 ± 16	1.01 ± 0.01	0.97 ± 0.01	6578 ± 43	1.63 ± 0.08 ^b
	3.1 (Large)	2.9 ± 0	-	3.81 ± 0.15	3.45 ± 0.34	1869 ± 198	1.62 ± 0.03 ^b
	Premix	-	-	56.4 ± 0.60	26.85 ± 0.90	237 ± 9	4.27 ± 0.55 ^{a*}
LCT	0.4 (Small)	53.4 ± 6.3*	455 ± 2	0.51 ± 0.01	0.48 ± 0.02	13586 ± 401	1.71 ± 0.09 ^b
	0.8 (Medium)	17.7 ± 1.3*	862 ± 23	0.98 ± 0.01	0.92 ± 0.01	7108 ± 82	1.83 ± 0.08 ^b
	3.1 (Large)	3.1 ± 0.2	-	3.51 ± 0.22	3.54 ± 0.33	1842 ± 18	1.73 ± 0.19 ^b
	Premix	-	-	64.6 ± 0.94	36.5 ± 0.64	178 ± 3	6.12 ± 0.26 ^{a*}

The values with different superscript letters in a set of data are significantly different ($p < 0.05$). Values with the same superscript letter are not significantly different ($p > 0.05$). * $p < 0.05$ vs the value obtained using SCT for each droplet size emulsions. “-” if the value was not measured.

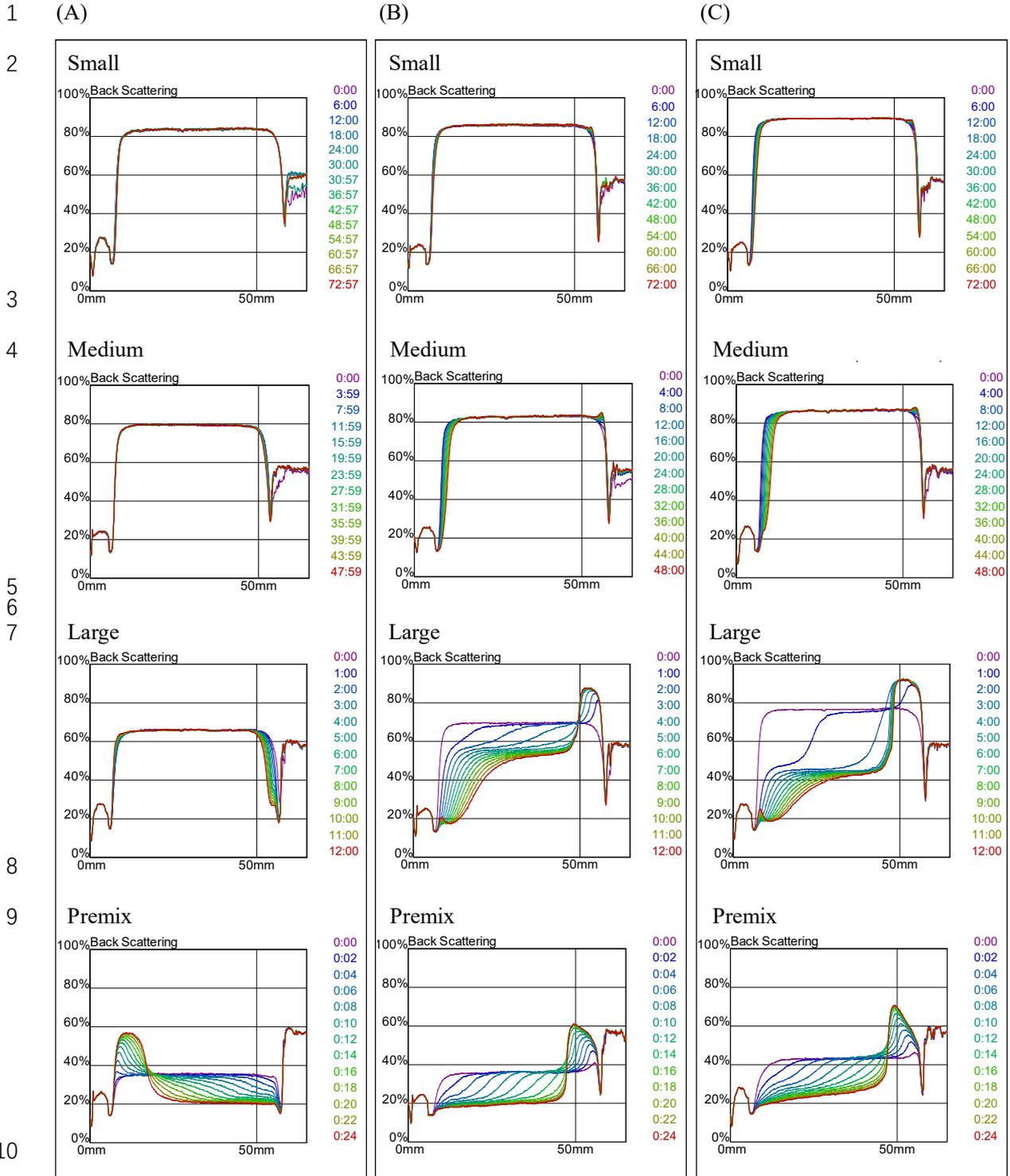
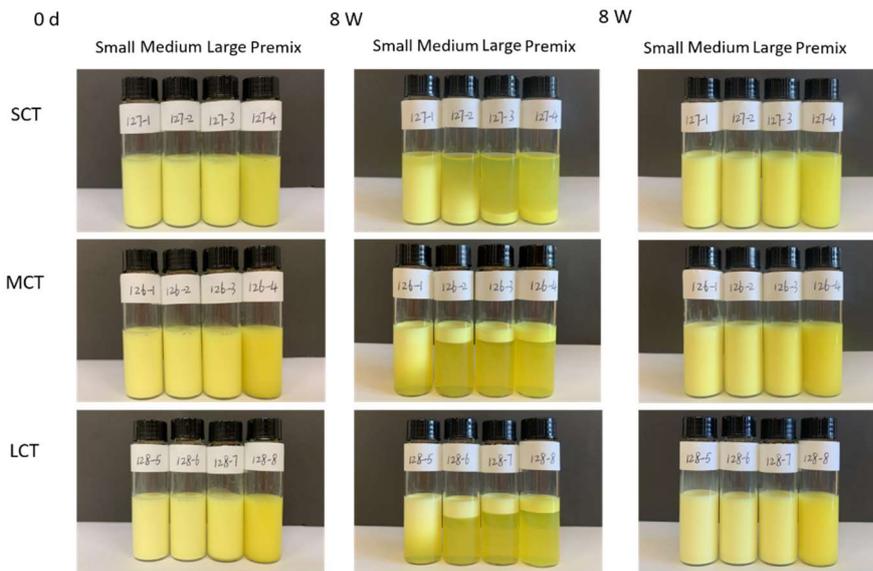


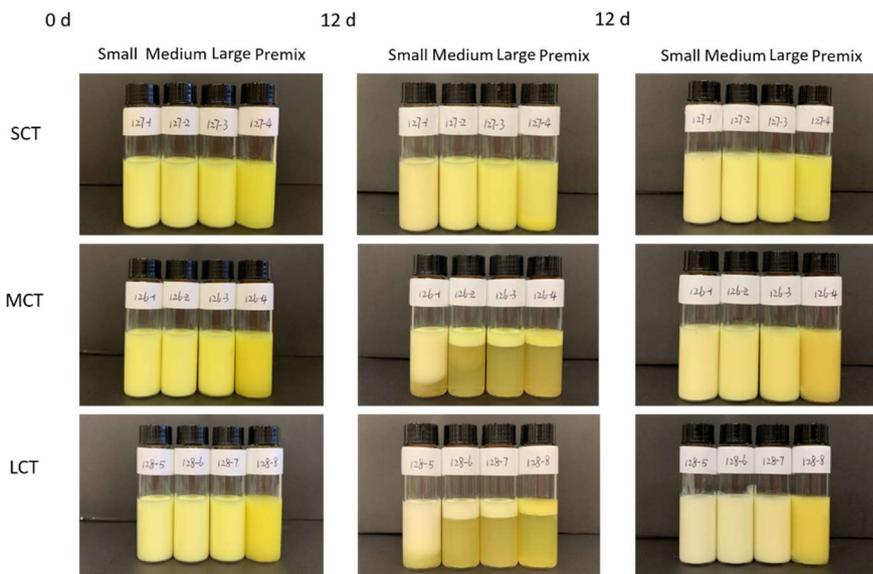
Fig. 1. Back scattering distribution (%) of different droplet size (Small, Medium, Large, and Premix, top to bottom) SCT (A), MCT (B) and LCT (C) emulsions at room temperature.

16 (A)



17

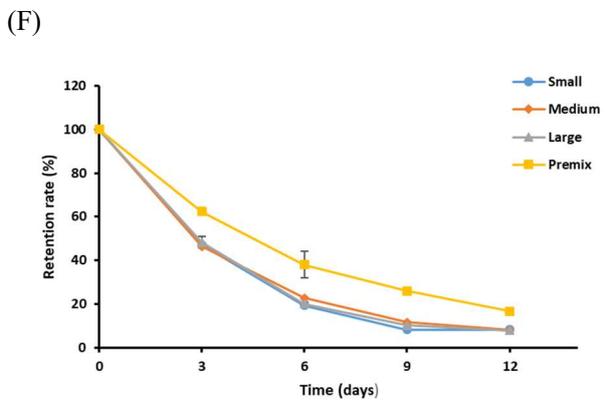
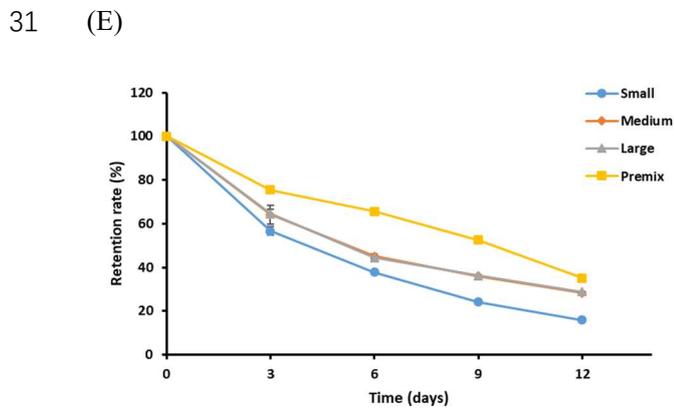
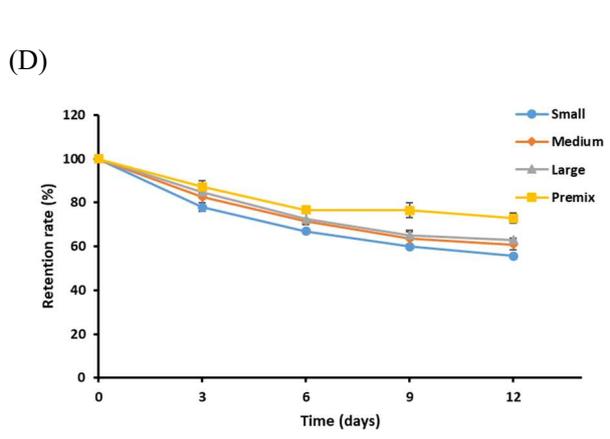
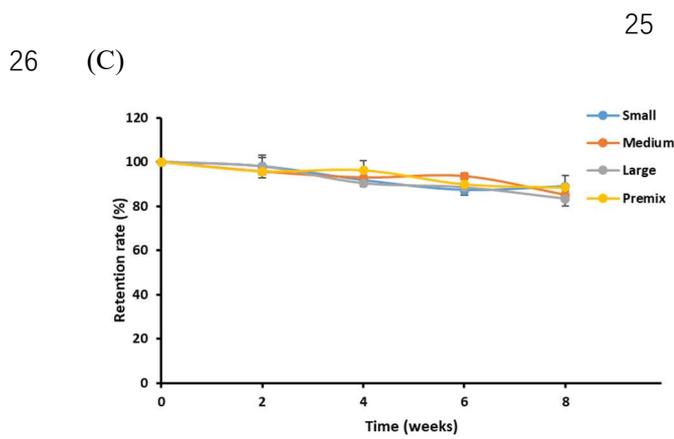
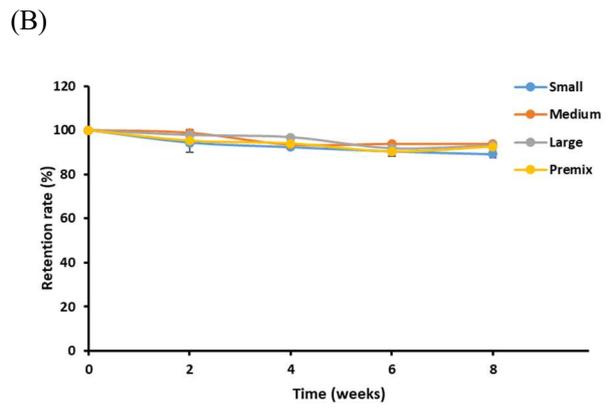
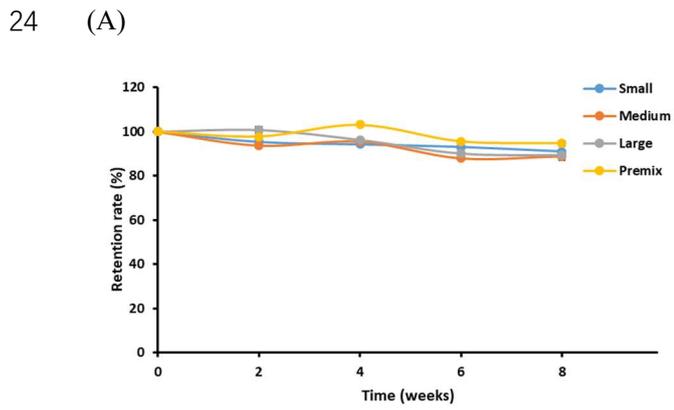
18 (B)



19

20 **Fig. 2.** Photographs of curcumin-loaded SCT, MCT and LCT emulsions with different droplet size (Small,
21 Medium, Large, and Premix, left to right) at the beginning (0 days) and at the end of storage (8 weeks at 25°C
22 (A), 12 days at 55°C (B)) in original and redispersed state (left to right).

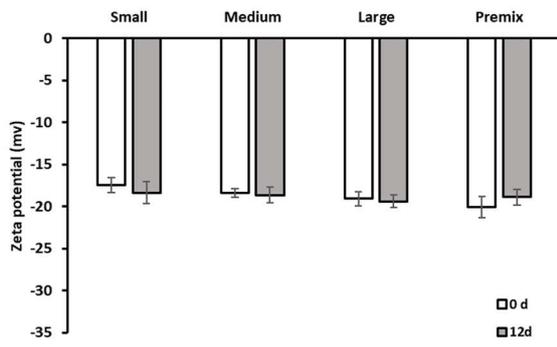
23



38 **Fig. 3.** Influence of emulsion droplet size (Small, Medium, Large, and Premix) on curcumin retention rate in
 39 SCT (A), MCT (B) and LCT (C) emulsions at 25°C and SCT (D), MCT (E) and LCT (F) emulsions at 55°C
 40 during storage.

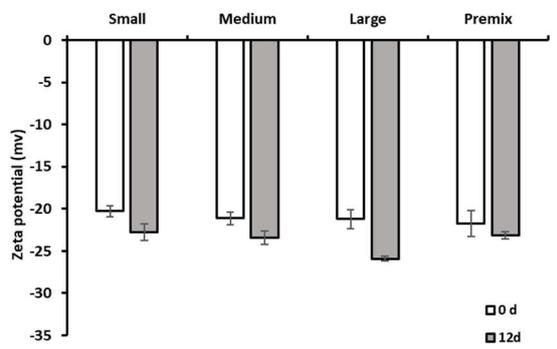
41

42 (A)



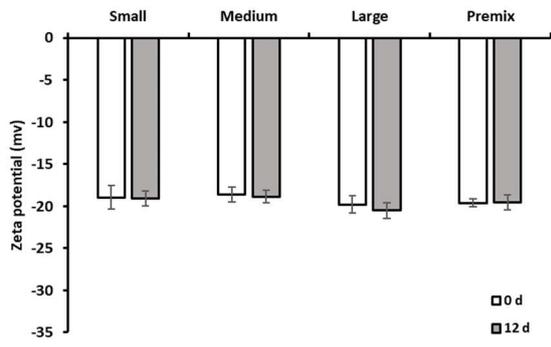
43

44 (C)

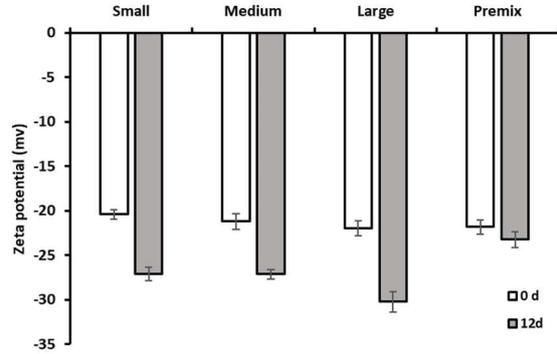


45

(B)



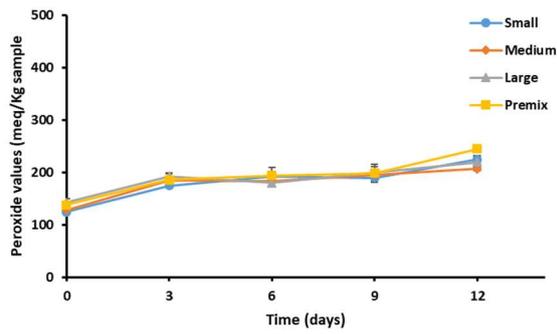
(D)



46 **Fig. 4.** Zeta potential change of curcumin-loaded SCT (A), MCT (B) and LCT (C) emulsions and LCT
47 emulsions without curcumin (D) before and after storage at 55°C for 12 days.

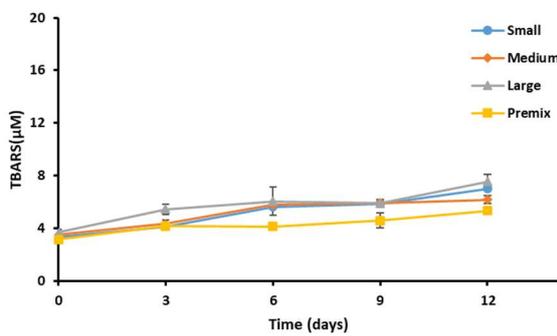
48

49 (A)



50

51 (C)



52

53 **Fig. 5.** Oxidation stability of LCT emulsions with and without curcumin during storage at 55°C. Peroxide
54 values of emulsions with (A) and without (B) curcumin; TBARS formation in emulsions with (C) or without
55 (D) curcumin.

56

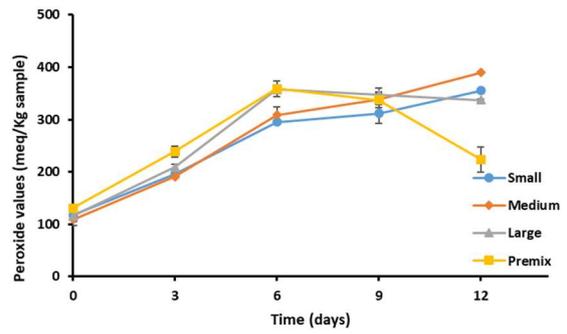
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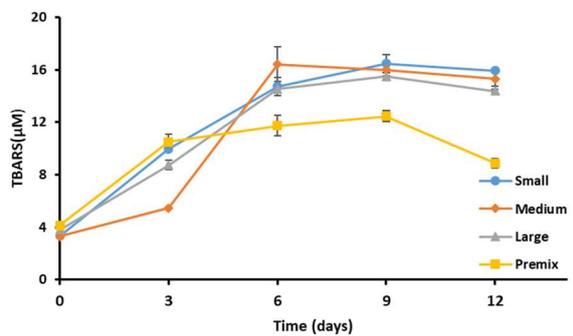
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60

(B)



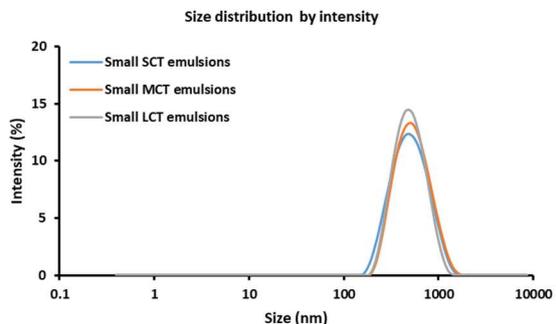
(D)



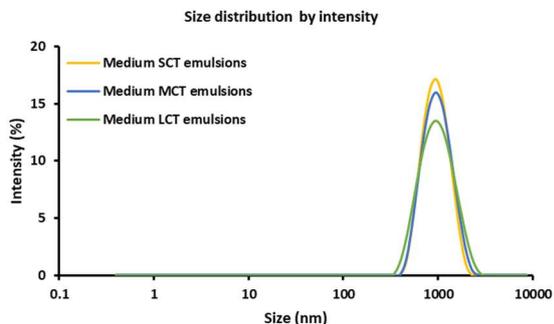
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62 **Supplementary materials**

63 (A)

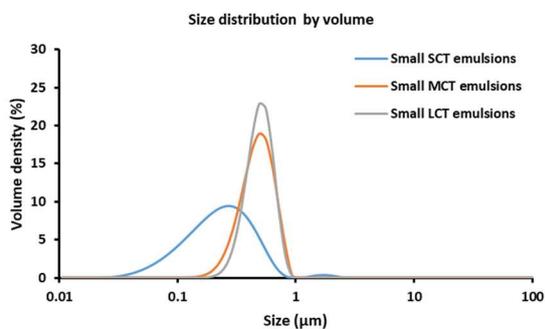


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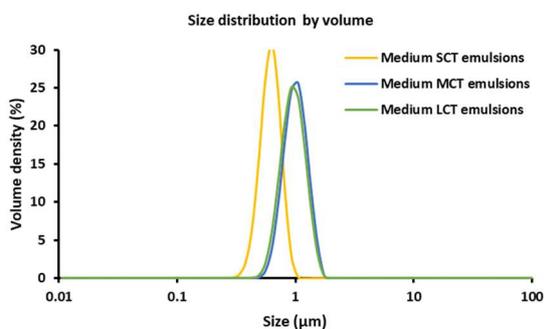


64

65 (C)

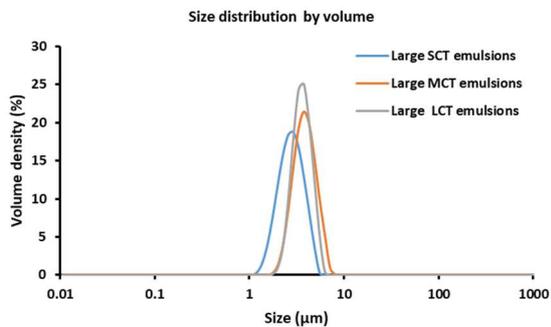


(D)

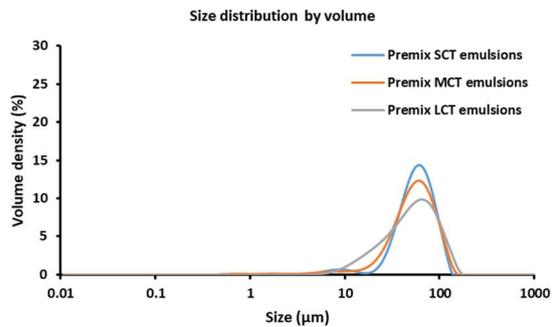


66

67 (E)



(F)



68

69 **Fig. S1.** Droplet size distribution of emulsion with different droplet sizes and different carrier
70 oils by intensity and volume. Size distribution of small (A) and medium (B) emulsion with
71 different carrier oils by intensity. Size distribution of small (C), medium (D), large (E) and
72 premix (F) emulsion with different carrier oils by volume.

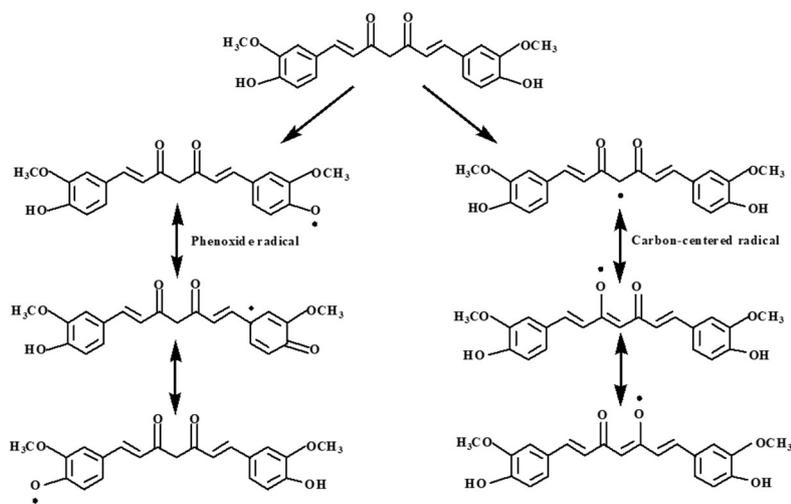
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A

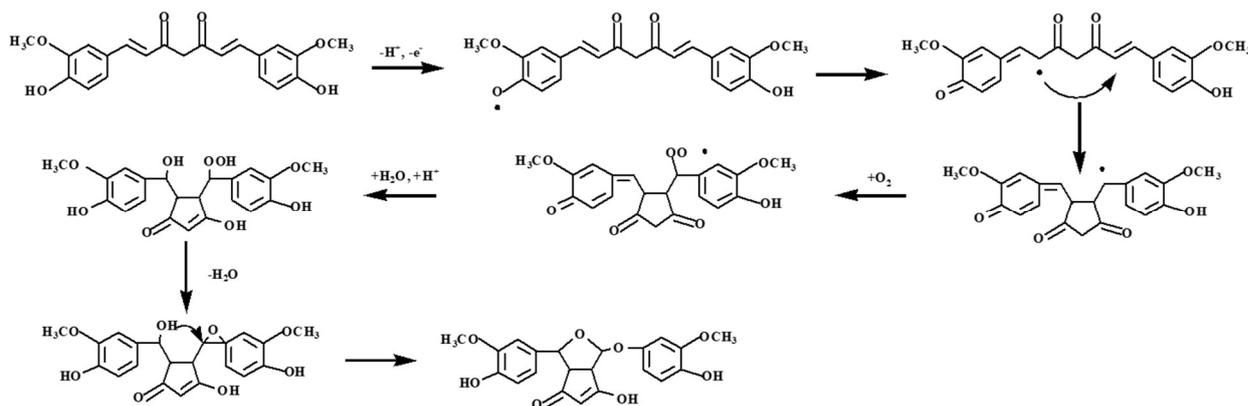


78

79

B

80



81

82 **Fig. S2.** Antioxidant mechanism of curcumin. (A): The mechanism of curcumin scavenging
83 free radicals (adapted from Del Prado-Audelo et al. (2019)). (B): The mechanism for the
84 autoxidation of curcumin (adapted from Salem et al. (2014)).

85

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